

MARTIUS YELLOW, SOLUTION

IVD *In vitro* diagnostic medical device



For use with Martius Scarlet Blue (MSB) kit

INSTRUCTIONS FOR USE

REF Product code: MAY-OT-30 (30 mL)

MAY-OT-100 (100 mL)

Introduction

Martius Yellow, solution is a component of Martius Scarlet kit; it is used for erythrocyte and fresh fibrin clusters visualization. Histology, cytology and other related scientific disciplines study the microscopic anatomy of tissues and cells. In order to achieve a good tissue and cellular structure, the samples need to be stained in a correct manner. Martius Scarlet Blue staining technique is used for fibrin visualization, especially of older clusters. This method is a modified Masson Trichrome method and it is ideal for studying connective tissue and vascular pathology.

Product description

- **MARTIUS YELLOW, SOLUTION** – Martius Yellow powder dye solution, optimal concentration

Example of staining with Martius Yellow, solution as a component of Martius Scarlet Blue kit

Other slides and reagents that may be used in staining:

- Fixatives such as BioGnost's neutral buffered formaldehyde solutions: Formaldehyde NB 4%, Formaldehyde NB 10%
- Dehydrating/rehydrating agent, such as BioGnost's alcohol solutions: Histanol 70, Histanol 80, Histanol 95 and Histanol 100
- Clearing agent, such as BioClear xylene or its aliphatic hydrocarbon substitutes, such as BioClear New
- Infiltration and fitting agent, such as BioGnost's granulated paraffin BioWax Plus 52/54, BioWax 56/58, BioWax Plus 56/68, BioWax Blue
- Covering agents for microscopic sections and mounting cover glass, such as BioGnost's BioMount, BioMount High, BioMount M, BioMount New, BioMount New Low, BioMount DPX, BioMount DPX High, BioMount DPX Low, BioMount C, or BioMount Aqua
- Glass slides used in histology, pathology and cytology, such as VitroGnost SUPER GRADE or VitroGnost COLOR, or one of more than 30 BioGnost's glass slides
- BioGnost's immersion media, such as Immersion oil, Immersion oil, types A, C, FF, 37, or Immersion oil Tropical Grade
- BioGnost's reagents that comprise Martius Scarlet Blue kit: Ponceau S, solution (PONS-OT-30, PONS-OT-100), Aniline blue, solution (ABO-OT-30, ABO-OT-100), Phosphomolybdic acid, 1% solution (FVK1-OT-30, FVK1-OT-100), Hematoxylin, Weigert A (HEMA-OT-30, HEMA-OT-100), Ferri reagent, Weigert B (FR-OT-30, FR-OT-100), Acid alcohol (KA-OT-100, KA-OT-250)

Preparing histological sections for staining

- Fix the tissue sample tightly (4% NB Formaldehyde, 10% NB Formaldehyde), rinse with water and dehydrate through series of ascending alcohol solutions (Histanol 70, Histanol 80, Histanol 95 and Histanol 100).
- Clear the sample with intermedium; in xylene (BioClear) or in a xylene substitute (BioClear New).
- Infiltrate and fit the sample in paraffin (BioWax 52/54, BioWax Plus 56/58, BioWax 56/58, BioWax Blue, BioWax Micro).
- Cut the paraffin block to **4-6 μm** slices and place them on a VitroGnost glass slide.

NOTE

Apply the reagent so it completely covers the section.

Sample staining procedure

a) using kit for 100 tests (MSB-100T)

1.	Deparaffinize the section in xylene (BioClear) or in a xylene substitute (BioClear New)	3 exchanges, 2 min each
2.	Rehydrate using 100% alcohol (Histanol 100)	2 exchanges, 5 and 3 min
3.	Rehydrate using 95% alcohol (Histanol 95)	2 min
4.	Rehydrate in distilled (demi) water	2 min
5.	Apply 5 drops of Hematoxylin, Weigert A and 5 drops of Ferri reagent, Weigert B. Gently stir and let it react	10 min
	Note: Working solution is stable for approximately 2 weeks. After the nuclei start turning brown, discard the working solution	
6.	Rinse the section quickly under tap water	
7.	Immerse into Acid alcohol, MSB	2 dips
8.	Rinse the section in tap water	5 min
9.	Rinse using 95% alcohol (Histanol 95)	2 min
10.	Add Martius Yellow solution (≥5 drops)	5 min
11.	Rinse in demineralized water	2 min
12.	Add Ponceau S solution (≥5 drops)	10 min
13.	Rinse in demineralized water	2 min
14.	Add Phosphotungstic acid, 1% solution (≥5 drops)	4 min
15.	Rinse in demineralized water	2 min
16.	Add Aniline Blue solution (≥5 drops)	5 min
17.	Rinse in demineralized water	2 min
18.	Dehydrate using 95% alcohol (Histanol 95)	2 exchanges, 30 seconds each
19.	Dehydrate using 100% alcohol (Histanol 100)	2 exchanges, 1 min each
20.	Clear the section in xylene (BioClear) or in a xylene substitute (BioClear New)	2 exchanges, 2 min each

Immediately after clearing apply an appropriate BioMount medium for covering/mounting on the section. If BioClear xylene was used, use one of BioGnost's mounting xylene-based media (BioMount, BioMount High, BioMount M, BioMount DPX, BioMount C, or universal BioMount New). If BioClear New xylene substitute was used, the appropriate covering agent is BioMount New. Cover the section with VitroGnost cover glass.

b) using seven-reagent 100 mL kit (MSB-K-100)

Pour the reagents into glass staining jars (Coplin, Hellendahl or Schifferdecker), return to original bottles after staining. Close tightly. Filter the reagents if necessary.

1.	Deparaffinize the section in xylene (BioClear) or in a xylene substitute (BioClear New)	3 exchanges, 2 min each
2.	Rehydrate using 100% alcohol (Histanol 100)	2 exchanges, 5 and 3 min
3.	Rehydrate using 95% alcohol (Histanol 95)	2 min
4.	Rehydrate in distilled (demi) water	2 min
5.	Prepare the Weigert hematoxylin working solution: mix equal volumes of Hematoxylin, Weigert A and Ferri reagent, Weigert B	10 min
	Note: Prepare the working solution of volume adequate for staining test sections. Working solution is stable for approximately 2 weeks. After the nuclei start turning brown, discard the working solution	
6.	Rinse the section quickly under tap water	
7.	Immerse into Acid alcohol, MSB	2 dips
8.	Rinse the section in tap water	5 min
9.	Rinse using 95% alcohol (Histanol 95)	2 min
10.	Immerse into Martius Yellow solution	5 min
11.	Rinse in demineralized water	2 min
12.	Immerse into Ponceau S solution	10 min
13.	Rinse in demineralized water	2 min
14.	Immerse into Phosphotungstic acid, 1% solution	4 min
15.	Rinse in demineralized water	2 min
16.	Immerse in Aniline Blue solution	5 min
17.	Rinse in demineralized water	2 min
18.	Dehydrate using 95% alcohol (Histanol 95)	2 exchanges, 30 seconds each
19.	Dehydrate using 100% alcohol (Histanol 100)	2 exchanges, 1 min each
21.	Clear the section in xylene (BioClear) or in a xylene substitute (BioClear New)	2 exchanges, 2 min each

Immediately after clearing apply an appropriate BioMount medium for covering/mounting on the section. If BioClear xylene was used, use one of BioGnost's mounting xylene-based media (BioMount, BioMount High, BioMount M, BioMount DPX, BioMount C, or universal BioMount New). If BioClear New xylene substitute was used, the appropriate covering agent is BioMount New. Cover the section with VitroGnost cover glass.

Result

- Nuclei - blue/black
- Muscles - dark red
- Fibrins - red (young clusters may be stained yellow, and older ones blue)
- Collagen - blue
- Erythrocytes - yellow

Note

Staining procedures are not standardized and they depend on standard operating procedures of individual laboratories and the experience of the personnel conducting the staining procedure. Intensity of staining depends on the period of immersion in the dye. Depending on personal requests and standard laboratory operating procedures, sample processing and staining can be carried out according to other protocols.

Preparing the sample and diagnostics

Use only appropriate instruments for collecting and preparing the samples. Process the samples with modern technology and mark them clearly. Follow the manufacturer's instructions for handling. In order to avoid mistakes, the staining procedure and diagnostics should only be conducted by authorized and qualified personnel. Use only microscope according to standards of the medical diagnostic laboratory. In order to avoid an erroneous result, a positive and negative check is advised before application.

Safety at work and environmental protection

Handle the product in accordance with safety at work and environmental protection guidelines. Used solutions and out of date solutions should be disposed of as special waste in accordance with national guidelines. Chemicals used in this procedure could pose danger to human health. Tested tissue specimens are potentially infectious. Necessary safety measures for protecting human health should be taken in accordance with good laboratory practice. Act in accordance with signs and warnings notices printed on the product's label, as well as in BioGnost's material safety data sheet.

Storing, stability and expiry date

Keep Martius Scarlet Blue kit in a tightly sealed original packaging at temperature of 15 to 25°C. Keep in dry places, do not freeze and avoid exposing to direct sunlight. Date of manufacture and expiry date are printed on the product's label.

References

1. John, D. B. (2009): Theory and Practice of Histological Techniques, 6th ed.,
2. Stefan, J. A. (1984): Simultaneous demonstration of connective tissue elastica and fibrin by a combined Verhoeff 's elastic - Martius - Scarlet - Blue trichrome stain, Stain Technology, 59(1): 1-5
3. Mariusz, G. et al. (2017): Combined orcein and martius scarlet blue (OMSB) staining for qualitative and quantitative analyses of atherosclerotic plaques in brachiocephalic arteries in apoE/LDLR ^{-/-} mice, Cross Mark, 147(6): 671-681
4. Lendrum, A. C. (1962): Studies on the character and staining of fibrin, J Clin Pathol., 15(5): 401-413

MAY-X, V1-EN1, 30 August 2022, KB/IŠP

	Refer to the supplied documentation		Storage temperature range		Number of tests in package		Product code		European Conformity	BIOGNOST Ltd. Medjugorska 59 10040 Zagreb CROATIA www.biognost.com	
	Refer to supplied instructions		Keep away from heat and sunlight		Valid until		Lot number		Manufacturer		
	For in vitro diagnostic use only		Keep in dry place		Caution - fragile						